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EXTRACTION OF CELLULOSE FROM BAMBOO USING PRETREATMENT AND DELIGNIFICATION

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ABSTRACT

Biomass pretreatment process requires lignocellulosic prior to delignification process, with the aim to break down the lignin in the bamboo become protective structures of lignocellulosic, as well as making cellulose or hemicellulose which is more easily in the delignification. The delignification process is used to remove compounds of lignin and pentos in bamboo, making cellulose becomes optimum.
Variables remain in pretreatmentprocess is bamboo powder 100 mesh size, the volume sulfuric acid as much as 200 ml, water volume 3 liters, and the stirring speed 200 rpm. The free variables consist of heavy bamboo 50-250 grams, delignification time is 30-150 minutes, and delignification temperature is 25-125(^{0}C). The early bamboo composition of cellulose levels 42.4-53.6 (%), lignin levels 19.8-16.6(%), pentosan levels 3.47-1.24(%), levels of extractive substances 4.5-9.9(%), water levels 15-20 (%), ash levels 1.24-3.47 (%), and SiO2 levels 0.1-1.78 (%). After pretreatment one obtained a reduction in lignin levels 20-25 (%) and pentosan levels on bamboo, and cellulose level was obtained after delignification process by 40-50 (%). Cellulose obtained in the process of pretreatment and delignification can be used for raw material in the process fermentation to obtain glucose.

Keywords: Bamboo, Cellulose, Delignification process, Pretreatment process

Introduction

Biomass from plants has been declared as an alternative raw material for gasoline fuel substitution in the form of bioethanol, bioethanol obtained from biomass and bioenergy crops has been proclaimed as one of the feasible alternative as gasoline fuel Demirbas *et al*. (2011). The technology for lignocellulose ethanol production relies mainly on pre-treatment, chemical or enzymatic hyhrolysis, fermentation and product separation or distillation. An appropriate pretreatment strategy is essential for the efficient enzyme hydroysis of lignocellulosic biomass as lignin hinders the saccharification process. Various pretreatment approaches have been exploited in the past such as acid or alkali pretreatment, hydrogen peroxide pretreatment, steam explosion, liquid hot water, ammonia fiber expansion pretreatment, sodium chorite pretreatment, and biological pretreatment Kuhar *et al*. (2009).

The research conducted to evaluate acid pretreatment from hydroside paper waste as material for bioethanol production, optimized sulfuric acid hydrolysis Dubey *et al*. (2012). Chemical pretreatment of lignocellulosic biomass with Sulphuric (H_2SO_4) and phosphoris (H_3PO_4) acids are widely used since they are relatively cheap and efficient in hydrolysing lignocellulose, though the letter gives a milder effect and is more benign to the environment. Hydrochloric (HCl) acid is more volatile and easier to recover and attacks biomass better than H_2SO_4 Demirbas *et al*. (2008), similarly, nitric acid (HNO3) posseses good cellulose to sugar

conversion rates Tutt *et al*. (2012). However, both acids are expensive compared to sulphuric acid. Pretreatment of lignocellulose has received considerable research globally due to its effluence on the technical, economic and enviromental sustainability of cellulose ethanol production. This paper reviews know and emerging chemical pretreatment methods, the combination of chemical pretreatment with other methods to inprove carbohydrate preservationreduce formation to degradation product, achieve high sugar yield at mild reaction conditions, reduce solvent loads and enzyme dose, reduce waste generation Edem *et al*. (2013).

Technical lignocellulosic residues, case of sugarcane and blue agave bagasses Iliana *et al*. (2016). Initiatives of the future for lignin in biomass, pretreatment technologies to separate the tree main biopolimers (cellulose, hemicellulose, and lignin) Nicolas *et.al*. (2011). Pretreatment for hydrogen and bioethanol production from olive oil waste products was ethanol yield 5.4 % treatment with 1.75 w/v sulphuric acid and heated it at 140 ^{o}C for 10 min Federico *et al*. (2016). Pretreatment followed with simultaneous saccharification and fermentation on bioconversion of microcrystalline cellulose Yun *et al*. (2015). A sustainable feedstock bioethanol production, cellulose hydrolysis was microwave irradition using hydrochloric acid as catalyst, fermentation with yeast (Saccharomyces cerevisiae), modest reaction conditions (2.38 M acid consentration), irradition time 7 min, and yield of 0.67 g glucose / g cellulose Indra *et al*. (2014). Elements contained in the lignocellulose biomass of the plants are usually used lignocellulose biomass, potential for bioethanol production globally. Agriculture (soft wood), forestry (hard wood), and industrial waste are a major lignocellulose biomass for bioethanol production. The lignocellulosic biomass is one of the potential main sources for economic bioethanol production globally. Agricultural, forestry (soft and hardwoods) and industrial wastes are the major lignocellulosic biomasses Limayem, *et al*. (2014). The lignocellulosic biomass for bioethanol production was developed using inhibitors-tolerant Saccharomyces cerevisiae, more than 4 % (w/w) ethanol consentration was achieved, which corresponded to 72.3 % theoretical yield of ethanol Hongzhang, *et al*. (2016). Bioethanol production using sodium hydroxide pretreated sweet sorghum bagasse without washing, ethanol theoretical yield from 44.06 ± 0.93% to 65.14± 0.91 % Menghui, *et al*. (2016).

The bioethanol production from lignocellulose biomass using process pretreatment, hydrolysis, fermentation and recovery of ethanol Sarkar *et al*. (2012), Balat *et al*. (2008). The research conducted by Sarkar *et al*. (2012). about bioethanol production from agricultural waste using PROFER pretreatment method. The purpose of dilute acid pretreatment is the removal of hemicelluloses and the recovery of the sugar component. Among all pretreatment methods, the acid pretreatment methods of biomass with dilute sulfuric acid has long been recognized as a critical step of removing the hemicellulosic fraction from the lignocellulosic substrate to economize the biological conversion of cellulosic biomass to ethanol Kuhad, *et al*. (2010). The research conducted by Thangavelu, etc (2014). about ethanol production from sago pith waste (SPW) using microwave hydrothermal hydrolysis catalyzed by carbon dioxide, resulted in higher energy saving compared to previous techniques in the absence of enzymes, acid or base catalyst.

Bioethanol production from paper fibre residue using diluted NaOH and the fermentation process with microorganism pinicillium chrysogenum and Saccharomyces cerevisiae. The fermentability of the hydrolysate decreased strongly for hydrolysate produced at temperature higher than 50 ^{o}C, the ethanol consentration of monosaccharide hydrolysate was found to be 34.06 g/L and ethanol yield was 0.097 g/g Lim, *et al*. (2012).

Bioethanol production from lignocellulosic biomass involves different step such as pretreatment, hidrolysis, fermentation and ethanol recovery Balat, *et al*. (2008). The technology for lignocellulosic ethanol production relies mainly on pre-treatment, chemical or

enzymatic hyhrolysis, fermentation and product separation or distillation. An appropriate pretreatment strategy is essential for the efficient enzyme hydroysis of lignocellulosic biomass as lignin hinders the saccharification process. Various pre-treatment approaches have been exploited in the past such as acid or alkali pretreatment, hydrogen peroxide pretreatment, steam explosion, liquid hot water, ammonia fiber expansion pretreatment Teymouri, *et al*. (2005).

Cellulosic or second generation (SG) bioethanol is produced from lignocellulosic biomass (LB) in three main steps: pretreatment, hydrolysis, and fermentation. Pretreatment involves the use of physical processes, chemical methods, physico-chemical processes, biological methods, and several combinations there of to fractionate the lignocellulose into its components. It result in the disruption of lignin seal to increase enzyme access to holocellulose Lim, *et al*. (2012), Pang, *et al*. (2008), reduction of cellulose crystallinity Gabhane, *et al*. (2011), Kim, *et al*. (2008), and increase in the surface area Lee, *et al*. (2008), Li, *et al*. (2004) and porosity Harmsen, *et al*. (2010), Lee, *et al*. (2011) of pretreated substrates, resulting in increased hydrolysis rate. In hydrolysis, cellulose and hemicelluloses are broken down into monomeric sugars via addition of acids or enzymes such as cellulase. Enzymatic hydrolysis offers advantages over acids such as low energy consumption due to the mild process requirement, high sugar yield, and no unwanted wastes. Enzymatic hydrolysis of cellulose affected by properties of the substrate such as porosity, cellulose fibre crystallinity, and degree of polymerization, as well as lignin and hemicellulose ontent Van Dyk, *et al*. (2012), Mc Millan, *et al*. (1997), optimum mixing Lavenson, *et al*. (2012), substrate and end-product consentration, enzyme activity, reaction conditions such as pH and temperature Radeva, *et al*. (2012), Sun, *et al*. (2002).

From the previous research, it was known that cellulose from bamboo resulted good cellulose. The aim of this research was to search material bamboo from Indonesia with pretreatment process, and delignification process to gain cellulose product with high level of cellulose. The originality of this research was the second generation that was bamboo, by using two processes (pretreatment and delignification) simultaneously, technical cellulose production with combination acid (H_2SO_4) and base (NaOH) level of 40-50 (%), degradation lignin levels 20-25 (%), and pentosan levels as the substitution material of glucose.

Experimental

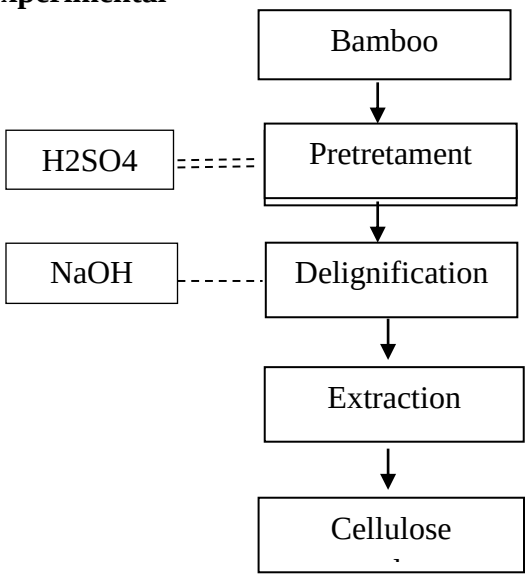

Fig.1 Cellulose production flow used pretreatment process, delignification process

From the result of laboratory analysis, it was known that bamboo forming elements were composition of cellulose levels 42.4-53.6(%), lignin levels 19.8-16.6(%), pentosan levels 3.47-1.24(%), levels of extractive substances 4.5-9.9(%), water levels 15-20 (%), ash levels 1.24-3.47 (%), and SiO2 levels 0.1-1.78 (%)

The cutting of bamboo with approximately 100 mesh size was done in order to obtain the high level of cellulose and low level of lignin during pretreatment process by H_2SO_4 and delignification process by NaOH. The quality of cellulose product was determined by various influencing parameter such as the ratio weight bamboo with volume H_2SO_4, ratio weight bamboo with volume NaOH, temperature, and delignification time.

The quality analysis of raw materials and cellulose product was done by laboratory analysis. The analysis was conducted on the instrumentation and gravimetric analysis by using Atomic Adsorbent Solvent (AAS) and Spectrophotometer, which analyzed items were the concentration of cellulose, lignin, pentosan, extractive substances, water, ash, and SiO2.

1.1. Procedure of Pretreatment and Delignification Process

Bamboo is dried under the Sun, weight bamboo is 50-250 grams, as many as 3 liter aquadest mixed with 200 ml of H_2SO_4 2N, then inserted into the tool extraction, in the heater with temperature 25-125 (^oC), and extraction time is 30-150 minutes. In this process, the lignin will separate from bamboo with filtering tools, so a layer of cellulose will be process hydrolysis with free lignin.

1.2. Procedure of Extraction Process

Sample of weight bamboo 50 grams, that has become powder added 3 L aquadest, and NaOH 2N with the variation of concentration of 2-10 (%v/v), then heated at a temperature 25-125 (^oC), then filtered and washed with water until neutral pH.

2. Results and Discussion

2.1 Quality Raw Materials

Bulrush using as a study material was derived from bamboo crops in the surrounding area. Assessment method was done by doing a survey and laboratory analysis to obtain some data about the quality and quantity of the available bamboo. The expected result was data about the quality and quantity of bamboo before processing to be an ethanol.

Based on the results of laboratory analysis, it was known that ethanol forming elements were cellulose, lignin and pentosan.

The cutting of bamboo with approximately length of 5 cm was done in order to obtain the high cellulose contain. Bulrush should be made in powder form, so cellulose can be hydrolyzed perfectly. However that process took an higher cost. Besides, bamboo in the powder form could suffer the physical destruction. The drying process of bamboo was naturally done first in the room temperature. The drying process was done in an oven at 100^0C for 3 hours. This was done for cost savings. The drying process aimed to reduce the water content in ethanol. Water level that was permitted by Standart National Indonesia (SNI) was 1%.

2.2 Extraction Process

In base extraction, sulfate acid was commonly used in certain level. Extaction was usually done in a special tank made of stainless steel or copper pipe connected to the heating ducts and exhaust pipes in order to regulate the air pressure (Kuhad *et al*., 2010). The cellulose

content of bulrush could be converted into glucose by concentrated base extraction process with certain concentration.

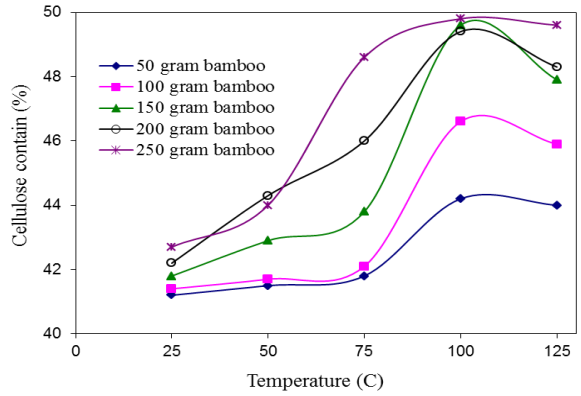

Fig.1 Changes cellulose contains with addition sulfate acid in bamboo and variation temperature extraction.

Figure 1 showed extraction process was done by the various weight of bulrush: 50 100, 150, 200, and 250 (grams) by the addition sulfate acid. After the extraction process was finished thus the solid and filtrate were obtained. The filtrate will be processed by the fermentation process to obtain ethanol concentration and solids can be used as compost. The effect of pH was essential in the fermentation process so filtrate must be measured for pH in the minimum level of 3.5 until the maximum level of 4.5. The optimum cellulose contain as 100 ^{O}C and 250 grams bamboo is 49 %.

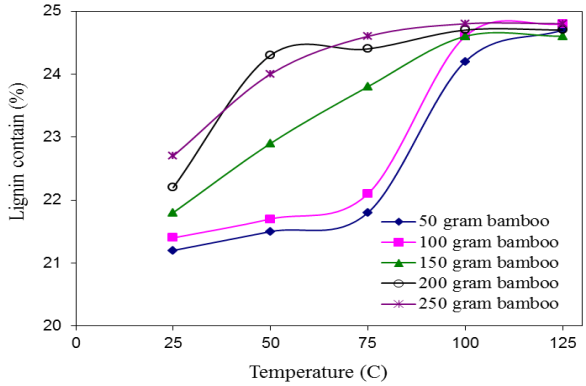

Fig. 2 Changes lignin contains with addition sulfate acid in bamboo and variation temperature extraction.

Figure 2 showed extraction process was done by the various weight of bulrush: 50 100, 150, 200, and 250 (grams) by the addition sulfate acid. After the extraction process was finished thus the solid and filtrate were obtained. The constan lignin contain as 100-125 (^{O}C) and some bamboo is 24.7 %.

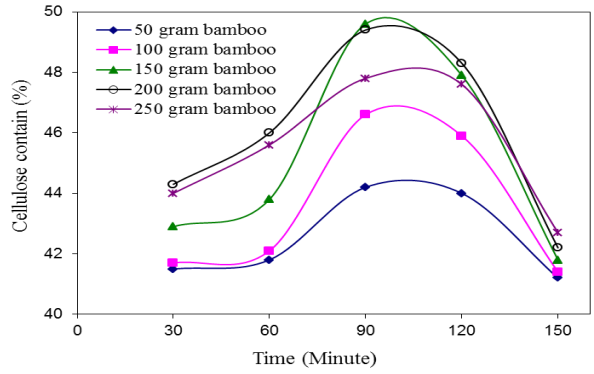

Fig. 3 Changes cellulose contains with addition sulfate acid in bamboo and variation time extraction.

Figure 3 showed extraction process was done by the various weight of bulrush: 50 100, 150, 200, and 250 (grams) by the addition sulfate acid. After the extraction process was finished thus the solid and filtrate were obtained. The filtrate will be processed by the fermentation process to obtain ethanol concentration and solids can be used as compost. The effect of pH was essential in the fermentation process so filtrate must be measured for pH in the minimum level of 3.5 until the maximum level of 4.5. The optimum cellulose contain as 90 minute and 150 grams bamboo is 49 %.

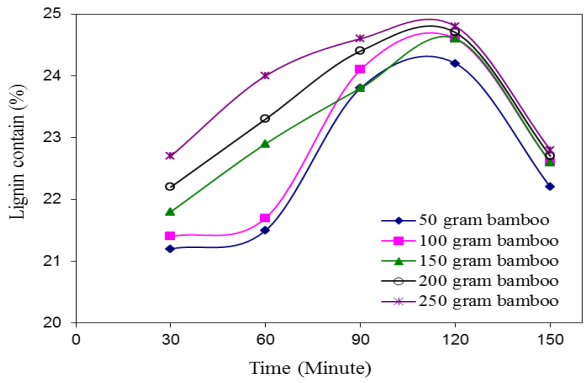

Fig. 4 Changes lignin contains with addition sulfate acid in bamboo and variation time extraction.

Figure 4 showed extraction process was done by the various weight of bulrush: 50 100, 150, 200, and 250 (grams) by the addition sulfate acid. After the extraction process was finished thus the solid and filtrate were obtained. The constan lignin contain as 90-120 (minute) and some bamboo is 24.7 %.

Conclusion

Based on the aim of research in reviewing of process pretreatment, delignifikasi and extraction, and also searching for alternative material of cellulose product. The optimum cellulose contain in the extraction process was 49%, and decreased lignin contain is 24.7%, at temperature 100 C and 90-120 (menit).

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